MEASURING THIAMINE DISULFIDE REDUCING SUBSTANCES IN CHEDDAR CHEESE[1]

The presence of hydrogen sulfide in Cheddar cheese and its relationship to desirable Cheddar flavor has been reported (2). Presumably, it is formed in cheese from sulfhydryl groups as a result of bacterial activity. Therefore, the question was raised concerning the quantity of sulfhydryl groups in cheese and possible methods for their detection. Attention was focused on the Thiamine Disulfide (TDS) method of Harland and Ashworth (1), which was adapted for use on cheese.

EXPERIMENTAL PROCEDURE

Procedure. The modifications of the original method involved the preparation and reaction of the cheese-substrate mixture, primarily. Preparation of the thiamine disulfide substrate, oxidation of the reduced substrate, and recovery and estimation of thiochrome were according to Harland and Ashworth (1).

One gram of cheese, taken from the solid portion of a plug at least 1 in. below the surface, was transferred quickly to a 20-ml test tube calibrated to 10 ml and containing 5 ml of 2% sodium citrate, 1 ml of thiamine disulfide substrate, and four drops of iso-butyl alcohol. The cheese was macerated with a glass rod and the mixture permitted to react at room temperature for 2 hr with intermittent stirring with the glass rod to facilitate complete suspension of the cheese. The reaction was stopped by addition of 10% TCA to 10 ml total volume. After centrifugation, the supernatant was filtered through a small wad of cotton and 1 ml of the filtrate diluted to 50 ml with distilled water. Two milliliters of this material was oxidized with potassium ferricyanide and the analysis continued as outlined (1). For the reagent blank, 2.9 ml of 10% TCA was added to the sodium citrate–TDS substrate mixture before the cheese was added to the test tube.

Allowances for cheese with relatively high or low TDS reducing capacities may be made by doubling the amount of cheese, altering the dilution of the cheese–TDS reagent filtrate, or varying the amount of solution to be oxidized within a range of from 1 to 5 ml.

Addition of from 0.2 to 2 mg cysteine · HCl to the cheese-substrate reaction mixture resulted in recoveries of 100% ± 2% cysteine · HCl. Less than 10% variation was observed between duplicate analysis of the same cheese. Concerning the latter, it is extremely important that solid portions from a freshly drawn plug or cut surface of cheese is used. Open portions of cheese have been observed to contain up to 50% less TDS reducing materials than solid portions.

TDS reducing materials in Cheddar cheese. To obtain information concerning the concentration of TDS reducing materials in Cheddar cheese, 23 samples of cheese ranging in age from one to 18 months were obtained from two commercial sources. The results (Table 1) indicate an over-all range of TDS materials from 2.3 to 30.2 mg cysteine · HCl equiv./100 g. Twenty-one of the samples had TDS values in excess of 10.0. The average TDS value for all of the samples was 15.9. With one exception, a one-month-old cheese with a TDS value of 2.3, the one- to 12-month-old cheese contained higher concentrations of TDS reducing compounds than the 16- to 18-month-old cheese, generally.

TABLE 1

Thiamine disulfide reducing compounds in commercial Cheddar cheese

No. of samples	Age	TDS (mg cysteine · HCl equivalent/100 g) Range	Average
	(months)		
10	1– 4	2.3–26.8	15.0
6	6–12	15.2–30.2	20.9
8	16–18	8.9–16.4	13.3

Although a number of compounds may contribute to the reduction of thiamne disulfide by Cheddar cheese, it is considered that the major portion of the reduction is caused by active sulfhydryl groups produced during the ripening process. H_2S reacts with thiamine disulfide also. The H_2S content of the present cheese was not determined, but based upon H_2S analysis of similar groups of commercial cheese (2, 3) it is estimated that the average contribution of H_2S to the TDS values of Cheddar cheese would be less than 2%.

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